Assemblies and Aggregates I

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A View to a Kill: T6SS-Mediated Cell Killing Visualized by Fluorescence Microscopy

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The Type Six Secretion System (T6SS) is a bacterial toxin-delivery system targeting bacterial cells which neighbor the donor, promoting recipient cell death. The T6SS is widely conserved among Gram-negative bacteria and may be a central determinant in bacterial fitness in polymicrobial communities of particular relevance to chronic infection. Sequence homology of secretion system components to the T4 bacteriophage tail spike, cryoEM reconstructions of the secretion system and fluorescence imaging are all consistent with a dynamic mechanism of secretion. The complex system, which is composed of at least 15 proteins, forms a puncturing apparatus/delivery system which uses a donor protein filament to puncture the recipient cell wall to deliver protein toxins. Using quantitative imaging analysis of multiple fluorescent fusions, we present a detailed characterization of T6SS system dynamics visualized in single cells in multiple bacterial species, developing a model of T6SS function. We present quantitative measurements of the dynamics of the secretion system - from the assembly to contraction to disassembly - in conjunction with quantitative measures of system function, including recipient cell lysis.

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A FRET-Based Method for Measurement of Yeast Septin Filament Formation In Vitro

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Septins are a family of GTP-binding cytoskeletal proteins conserved in all eukaryotes (except higher plants) that have roles in erecting diffusion barriers, affecting membrane curvature, and providing protein scaffolding. Budding yeast (Saccaromyces cerevisiae) is a convenient organism for investigating septin organization and function because of the wide variety of tools available. Because septins are conserved, understanding the principles that dictate how the yeast proteins organize should provide insight into septin structure and function in higher eukaryotes, including humans. Single-particle EM analysis has revealed that septin subunits associate to form linear apolar hetero-octameric rods. In vitro, septin rods polymerize end-on-end into long, straight paired filament. However, no facile method has existed for studying the polymerization of septin hetero-octamers in vitro in real time. We have previously generated yeast septin complexes wherein all endogenous Cys residues were eliminated by site-directed mutagenesis, except either one native Cys (C43) or an introduced E294C mutation in Cdc11. Because Cdc11 is the terminal subunit in hetero-octamers, derivatization of the single Cys residue with organic dyes should permit the use of Förster resonance energy transfer (FRET) to monitor filament assembly. In initial experiments conducted with mixtures of donor- and acceptor-labeled hetero-octamers, the expected FRET is exhibited under conditions that favor polymerization (low salt), but not under conditions known to prevent polymerization (high salt). Furthermore, the amount of FRET observed depends on the input concentrations and relative ratios of the donor and acceptor dye-labeled rods. These findings are fully consistent with previous observations made by EM and indicate that the observed FRET is providing a reliable solution-based assay for the end-to-end assembly of hetero-octamers into filaments. This assay can now be exploited to study the effects of septin concentration, cofactors (e.g., guanine nucleotides) and septin-interacting proteins.

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Investigating the Mechanism of Collagen Self-Assembly with Microrheology

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Collagen is the predominant protein in vertebrates, where it comprises more than 1/4 of the total protein and performs structural and mechanical tasks in the extracellular matrix and connective tissues. Formed intracellularly as a triple helical protein, collagen undergoes self-assembly extracellularly into fibrils, which in turn contribute to higher-order structures. Self-assembly of collagen into well-ordered fibrils can be replicated with appropriate solvent conditions *in vitro*, implying that the keys to self-association are found within its sequence.

We perform microrheology experiments on collagen systems, using optically trapped particles as probes of local, microscale viscoelasticity. Our initial experiments investigated the time-dependent development of viscoelastic heterogeneity in collagen systems as they undergo self-assembly from proteins in solution into fibrillar structures.[1] Here, we examine alterations in collagen's chemical composition that influence its self-assembly. We find that the removal of collagen's nonhelical "telopeptide" ends significantly reduces elasticity of collagen solutions at timescales from ~10 msec to ~1 sec. While in the acidic solutions of these experiments collagen does not assemble into fibrils, our results nonetheless provide insight into the catalytic mechanism of these short domains on fibril formation. The removal of telopeptides has long been known to slow down fibril formation, and telopeptides have previously been postulated to transiently associate with other chains in solution,[2] thus providing "docking points" from which lateral assembly of collagen triple helices into fibrils can proceed. Here, our microrheology experiments provide direct evidence of increased strength and duration of interprotein contact arising from the presence of telopeptides, critical in catalyzing self-assembly of fibrillar collagen systems.

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Atomic Force Microscopy Imaging Reveals Structural and Mechanical Properties of Dissociated Hemocyanins by Temperature

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Hemocyanins are protein complexes responsible for the oxygen transport in arthropods and molluscs. These proteins contain a copper binuclear active site and are assembled as four hexamers with an overall size around 1,7MDa. Using Atomic force microscopy (AFM) imaging on purified hemocyanins from Grammostola rosea and Paraphysa sp, we have observed that both species showed a similar tetrameric assembly and molecular volume. Given that Paraphysa sp survives at lower temperatures than Grammostola rosea we also study by AFM the dissociated hemocyanin complexes obtained by freeze-defreeze cycles. The gel filtration pattern of these complexes indicates a similar behaviour between both species. In addition, by force spectroscopy measurements on each hexameric component, we have determined their elasticity behaviour. Altogether these demonstrate that AFM is a powerful tool to record simultaneously both kinetics and mechanical properties of hemocyanin. Funded by Fondecyt Grant 1120169, Millennium Nucleus Grant P10-035F and Anillo Grant ACT1108.

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Tripeptides Screening Report: Proline is Important for Aβ Fibrils Depolymerization

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Alzheimer's disease (AD) is the most frequent form of dementia among the elderly and is associated with the extracellular A-beta amyloid deposits in brain. One of the most straightforward approaches for finding a treatment of Alzheimer's disease is targeting of Aβ amyloid fibrillization. In our work we performed comprehensive study of the effect of all possible three-amino acid peptides (8000 tripeptides in total) on Aβ fibrils depolymerization using the molecular modeling and we analyzed the binding affinity of tripeptides to Aβ fibrils. By both docking and MM-PBSA methods tripeptides containing Proline and aromatic amino acids were identified as potentially the most effective. The ability of selected tripeptides to destroy amyloid fibrils was also investigated experimentally by ThT fluorescence assay and AFM microscopy in vitro. Using in silico and in vitro methods we have showed that selected tripeptides can destroy preformed Aβ fibrils.